Annual Report – FY2000

Support of monitoring activities and site characterization at Grays Reef National Marine Sanctuary

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Submitted to:

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Introduction

In April 2000, the National Centers for Coastal Ocean Science (NCCOS) initiated a new project in cooperation with the National Marine Sanctuary Program: Support of Monitoring Activities and Site Characterization at Grays Reef National Marine Sanctuary (GRNMS). Three NCCOS Centers are involved in the work: the Center for Coastal Fisheries and Habitat Research (CCFHR), the Center for Coastal Environmental Health and Biomolecular Research (CCEHBR) and the Center for Coastal Monitoring and Assessment (CCMA).

Nine objectives were defined in the original, three year proposal. A status of work related to each objective is provided below. An opportunity to conduct coral biomarker research arose and as a result, status of work related to a 10th objective is included.

Participate in Grays Reef National Marine Sanctuary fish monitoring activities including work in adjacent deeper areas

Staff of CCFHR have been involved in fish monitoring efforts since initial baseline work in the 1980's. CCFHR staff continued to participate in the semi-annual fish monitoring efforts in the 1990's. Three fish monitoring surveys were conducted at GRNMS with the assistance of NCCOS personnel during FY00: April 2000 from the NOAA Ship FERREL, June 2000 from the NOAA Ship Jane Yarn and July 2000 from a smaller vessel from GRNMS. In April, all 20 fixed sites within GRNMS were visually censused. In June only four fixed sites were visually censused. In July, all 20 fixed sites were again visually censused.

Data from the April survey has been combined with data collected from 1995 to present and released most recently as Grays Reef Monitoring Data version 3. Data from the June and July censuses have been computerized and are currently being checked for accuracy. Grays Reef Monitoring Data version 4 will include all data collected through October 2000.

The opportunity to work in deeper water was not realized in FY2000 partially owing to dedication of shiptime and resources to other activities deemed of higher priority and partially owing to weather affecting our ability to work offshore when shiptime was available. During FY2001, working in deeper areas in the vicinity of GRNMS will receive greater priority.

Analyze fish monitoring data for changes in abundance and species composition over time (1995-1999)

Fish monitoring data from 1995 to April 2000 has been computerized, standardized and taxonomic issues have been resolved. Preliminary examinations of the data have been conducted. There are indications of temporal changes in species abundances over time (Figure 1) with some species showing no change (slippery dick, belted sandfish), some exhibiting decreases (gag, spottail pinfish) and some indicating increases (black sea bass). These patterns, however, are based on a preliminary examination of the data.

A meeting was held between Dave Colby (CCFHR), Jon Hare (CCFHR) and Dave Score (GRNMS) on 8 September 2000 to discuss the statistical approach for analyzing the fish monitoring data. Dave Score will take the lead on the analysis of the fish monitoring data for

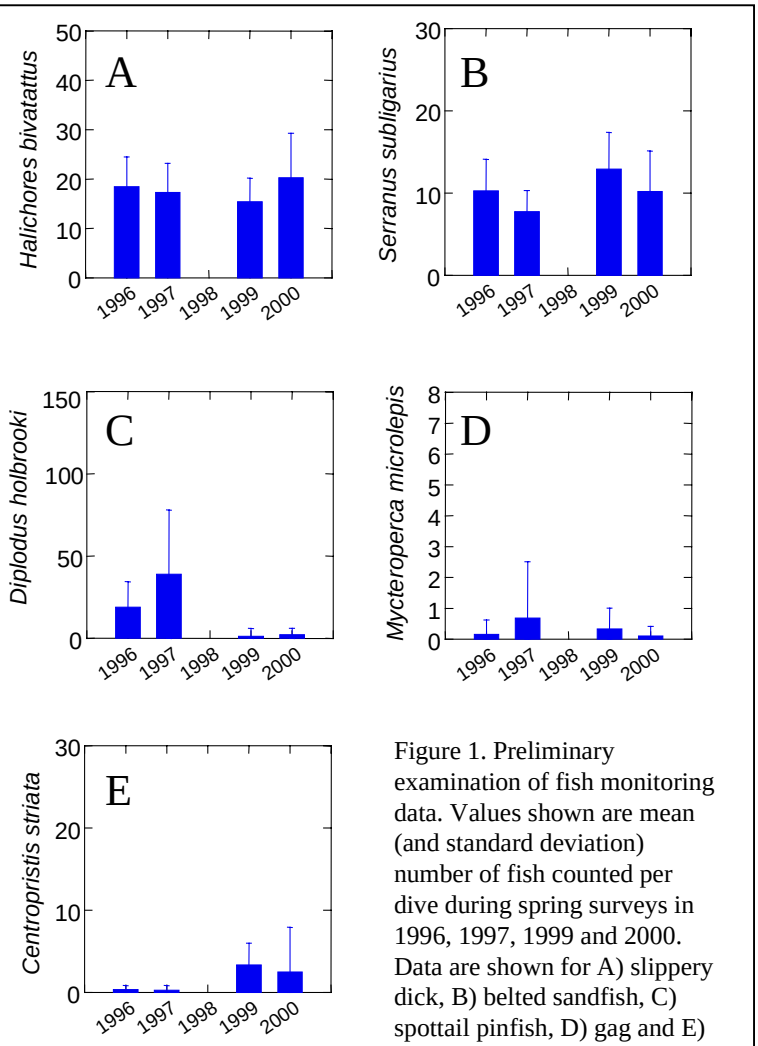

Figure 1. Preliminary examination of fish monitoring data. Values shown are mean (and standard deviation) number of fish counted per dive during spring surveys in 1996, 1997, 1999 and 2000. Data are shown for A) slippery dick, B) belted sandfish, C) spottail pinfish, D) gag and E) black sea bass.

changes in abundance and species composition over time. Initial steps will involve defining variability among divers and sites. The role of season and interannual variability in structuring the fish community at Grays Reef will then be examined. Dave Score will complete the analysis of these data as part of his Masters work at Georgia State University. Dave Colby and Jon Hare will provide advice on data analysis and interpretation. Dave Score has edited Grays Reef Monitoring Data version 3 resolving several taxonomic issues and prepared the dataset for analysis. Results from these analyses will be presented at the Fish Monitoring Workshop to be held in early 2001.

Assess adequacy of fish monitoring sampling design for detecting changes in abundance and composition of fishes over time

Progress on this objective depends on completion of the above objective and is thus tied to Dave Score finishing his Masters research. Based on a preliminary examination of the monitoring data, a broad outline of a modeling approach has been developed and will be pursued in FY2001.

Determine the importance of non-reef habitats to juvenile stages of reef fishes and evaluate the linkages between non-reef and reef habitats

Two field components were designed to address this objective: ROV characterization of benthic habitats and beam trawl sampling of juvenile fish in different benthic habitats. Three research cruises have supported this objective and cruise reports have been distributed:

http://www.bea.nmfs.gov/grnms/ . Approximately 225 minutes of video was taken with the ROV during the April FERREL cruise at eight of the 10 fixed beam trawl stations (Figure 2). These tapes have been previewed but not analyzed.

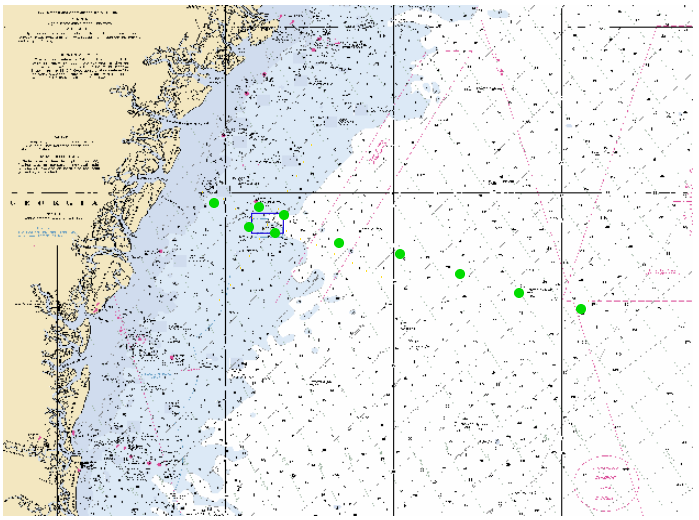

Figure 2. Location of ROV, beam trawl and broad-scale ichthyoplankton stations.

Table 1. Status of beam trawl sample processing.

Dates	Total	Sorted	Pre-ID	Final ID	Computerized
Apr-00	28	17	17		17
Jun-00	27				
Aug-00	27				

Beam trawl samples have been taken along a cross-shelf transect that bisects GRNMS (Figure 2). Four stations are located around the perimeter of the Sanctuary. Status of these samples is provided in Table 1. A wide variety of fishes inhabit the open sand areas in the vicinity of GRNMS (Table 2). Left-eyed flounders, toungefishes, cusk eels, sea robins and lizardfish were common. Very few reef-associated fishes have been collected, but only samples from April 2000 have been worked up. Reef-associated fishes were more common in June and August collections.

Provide customized satellite-derived sea surface temperature products to assist research and management activities within Grays Reef National Marine Sanctuary

Much progress has been made on customized SST imagery for GRNMS. Based on this work a customized product was provided to the Monitor National Marine Sanctuary to support their research during summer 2000: http://www.bea.nmfs.gov/monitor/. Although this product was not part of the original proposal, it was produced for the Monitor NMS owing to an expressed need. A similar initial product should be ready for GRNMS by October 31. The plan is to post images as they are received on the Southeast CoastWatch webpage. Users could then access this webpage from a link provided on the GRNMS web page. Images would be updated twice to four times daily.

Table 2. Summary of beam trawl during April 2000. All identifications are tentative. Approximately half of the samples collected in April 2000 are included here.

Species	Common Name	No.
Etropus microstomus	smallmouth flounder	305
Ophidion selenops	moon eye cuskeel	177
Porichthys plectrodon	Atlantic midshipman	166
Prionotus carolinus	Northern sea robin	143
Serraniculus pumilio	pygmy bass	57
Symphurus minor	largescale toungfish	50
Ophidion holbrooki	bank cuskeel	39
Monacanthus hispidus	planehead filefish	34
Unknown flounder	unknown flounder	22
Synodus foetens	inshore lizard fish	14
Diplectrum formosum	sand perch	11
Sygnathus fuscus	pipefish	10
Symphurus urospilus	spottail toungefish	10
Citharichtys macrops	spotted whiff	7
Lepophidium spp.	cusk eels	7
Sygnathus spp	pipefishes	6
Centropristis striata	black sea bass	5
Conger oceanicus	conger eel	5
Sparidae	porgy	5
Stenotomus spp	porgy	4
Ophichthys ocellatus	palespotted eel	3
Urophycis spp	hake	3
Cosmocampus profundus	deepwater pipefish	2
Hippocampus erectus	lined seahorse	2
Hypleurochilus geminatus	crested blenny	2
Peprilus triacanthus	butterfish	2
Prionotus spp	sea robin	2
Anchoa spp	anchovy	1
Anguilliform	eel	1
Ariosoma balearieum	bandtooth conger	1
Bothus spp	peacock flounder	1
Diplogrammus pauciradiatus	spotted dragonet	1
Engraulis eurystole	anchovy	1
Gobiidae	goby	1
Gobiosoma bosci	naked goby	1
Hildebrondia flava	yellow conger	1
Sygnathus elucens	pipefish	1
Symphurus diomedeanus	toungefish	1

Determine the species of fish that spawn in the vicinity of Grays Reef National Marine Sanctuary

Evaluate larval transport to and dispersal from Grays Reef National Marine Sanctuary to surrounding areas

These two objectives were examined using a combination of ichthyoplankton collections, CTD casts and drifter deployments. Three cruises have been conducted in support of these objectives and cruise reports have been distributed: http://www.bea.nmfs.gov/grnms/ .

In addition, Jon Hare (CCFHR) has had preliminary discussions with Cisco Werner (UNC-Chapel Hill), a South Atlantic Bight Synoptic Offshore Observational Network (SABSOON) PI (http://www.skio.peachnet.edu/projects/sabsoon.html) regarding potential collaboration to model larval transport.

Understanding black sea bass (*Centropristis striata*) spawning and larval transport is a top priority owing to its commercial and recreational importance. However, *Centropristis* larvae cannot be identified to species. Heart tissue of 8 adult *Centropristis striata* and 6 adult *Centropristis ocyurus* was collected by CCFHR staff following CCEHBR Marine Forensics Protocols. Tissue was transferred to CCEHBR and DNA has been extracted and amplified. Sequencing and analysis will be conducted in FY01. DNA from larval *Centropristis* will then be

extracted, amplified and sequenced allowing for the larvae to be identified to species. Once larvae can be identified to species, analysis of species-specific data can begin.

Broad-scale cross-shelf stations have been sampled with beam trawl stations (see Figure 2) and fine-scale along-shelf and cross-shelf stations have been sampled. Broad-scale stations were sampled in April and August 2000; fine-scale stations were sampled in April 2000. (Status of collections is provided in Table 3. Processing of April samples continues, but a wide diversity of larval fish have been collected (Table 4). A large number of larvae of reef associated species have been collected, primarily gobies and black sea bass/bank sea bass/sand perch larvae. The genetics work described above will assist in

Table 3. Status of ichthyoplankton sampling processing

Date	Total	Gear	Sorted	Pre-ID	final ID	Computerized
Apr-00	50	Bongo	35	35		35
Jun-00	0					
Aug-00	7	Sled				

Table 4. Summary of ichthyoplankton collections made in April 2000. All identifications are tentative.

Scientific ID	Number	Common Name	Adult Habitat
Gobiidae	474	**goby**	**reef**
Serraninae	108	**black sea bass/bank sea bass/sand perch**	**reef**
Sparidae	15	**porgy**	**reef**
Blenniidae	12	**blenny**	**reef**
Gobiesocidae	3	**clingfish**	**reef**
Halichoeres	2	**wrasse**	**reef**
Apogonidae	1	**cardnial fish**	**reef**
Triglidae	129	**sea robin**	**open sand**
Ophidiinae	105	**cusk eel**	**open sand**
Etropus	90	**smallmouth flounder**	**open sand**
Synodontidae	40	**lizardfish**	**open sand**
Diplogrammus pauciradiatus	23	**dragonet**	**open sand**
Leiostomus xanthurus	5	**spot**	**open sand**
Menticirrhus	4	**kingfish / sea mullet**	**open sand**
***Bothus* sp**	1	**peacock flounder**	**open sand**
Ophichthus	1	**snake eel**	**open sand**
Uranoscpidae	1	**stargazer**	**open sand**
Xyrichthys	1	**razorfish**	**open sand**
Engraulidae/Clupeidae	254	**anchovy/herring**	**water column**
***Peprilus* sp.**	2	**butterfish**	**water column**
Gonostomatidae	1		**water column**
Spheroides	22	**puffer**	**mixed/other**
Monocanthus hispidus	6	**planeheaded filefish**	**mixed/other**
Orthopristus crysoptera	4	**pigfish**	**mixed/other**
Sygnathus	4	**pipefish**	**mixed/other**
Cynoscion regalis ?	3	**weakfish**	**mixed/other**
Gerridae	3	**morjarra**	**mixed/other**
Larimus	1		**mixed/other**
Leptocephalus	1	**eel**	**mixed/other**
Micropogonias undulatus	1	**croaker**	**mixed/other**

identifying the latter group to species. A fair number of larvae of open sand species have also been collected including cusk eels and sea robins. Finally a number of small anchovies / herrings have been found. The results so far indicate that a number of fish species spawn in the vicinity of GRNMS. The large number of fish eggs that have been collected supports this statement; in 35 samples 11,183 eggs have been enumerated.

CTD data were collected in combination with beam trawl and ichthyoplankton sampling. Seasonal warming and increases in salinity are the most obvious signals seen in temperature and salinity data from April, June and August (Figure 3 and 4). Cross-shelf structure in

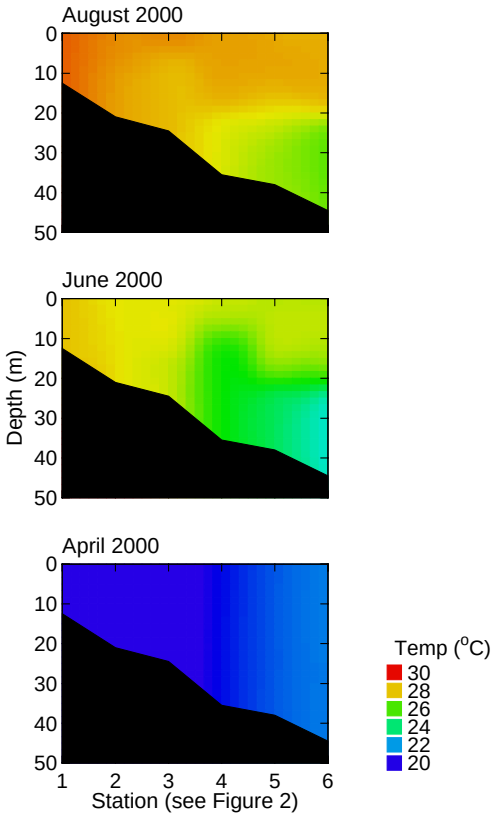

Figure 3. Profiles of cross-shelf temperature from three time periods during 2000. GRNMS is located at station 2 (station locations shown in Figure 2).

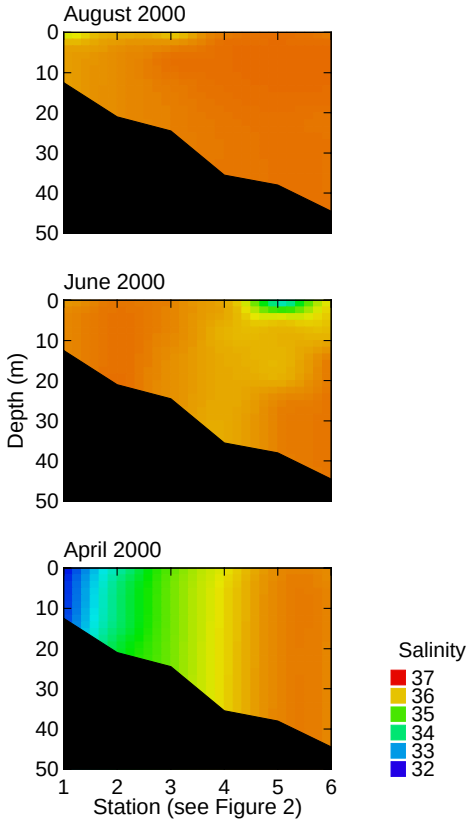

Figure 4. Profiles of cross-shelf salinities from three time periods during 2000. GRNMS is located at station 2 (station locations shown in Figure 2).

temperature and salinity is also apparent. However, in April there is little vertical structure in temperature and salinity, but in June and August vertical structure is apparent. The cooler and saltier water, observed deeper and offshore may indicate upwelling from the Gulf Stream but a more careful examination of the data is needed. In addition, analyses of ichthyoplankton and hydrographic data will elucidate the role of water mass structure in affecting larval fish distributions.

Table 5. Summary of drifter releases.

Date	Deployed	Processed	Days Tracked
Apr-00	3	3	60
Jun-00	3	3	60
Oct-00	3		15

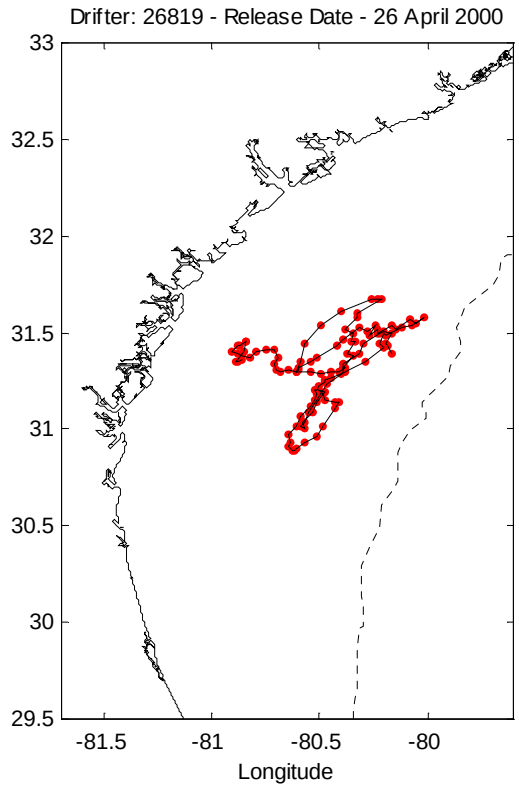

Figure 5. Track of a drifter released in April 2000. Shoreline and 50 m isobath are shown. Drifter was released within GRNMS.

Satellite-tracked drifters were released in combination with ichthyoplankton and CTD sampling. Six drifters were released within GRNMS in FY00: three in April and three in June. Three drifters were released in GRNMS in the beginning of FY01 (Table 5). Drifters released in April initially moved offshore, then exhibited large along-shelf movements (Figure 5). These movements appear to be related to wind forcing (Figure 6), but additional analyses are required. Wind data needs to be low-passed filtered and analyses need to be

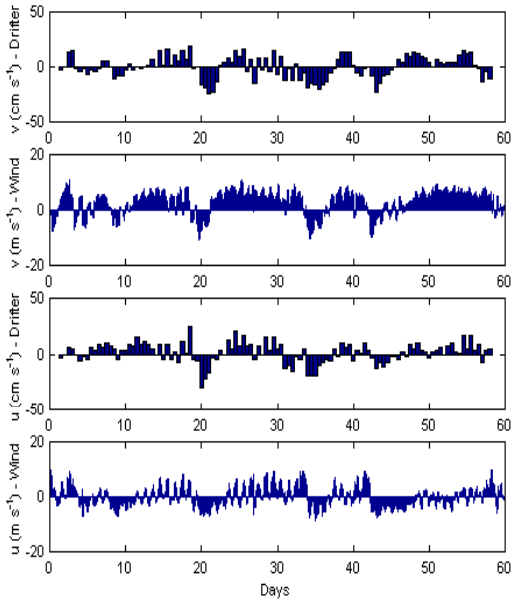

Figure 6. Comparison of north/south and east/west components of drifter 26819 movement with north/south and east/west component of the wind from NOAA buoy located in GRNMS.

conducted on the along -shelf and cross-shelf components of motion rather than the north/south and east/west components. Calculating along-shelf and cross-shelf components will require a spatial model of angles of rotation.

Drifters released in June moved northeastward along the shelf (Figure 7). Two of the drifters were picked up at sea and returned after 10 and 20 days respectively. The remaining drifter moved northeastward to Frying Pan Shoals and then moved offshore. It became entrained in the Gulf Stream and moved rapidly to the northeast. Velocities initially corresponded to winds observed at GRNMS. The relation between wind and drifter movement breaks down as the drifter is entrained into the Gulf Stream. Analyses of along-shelf and cross-shelf components of motion are required as described above.

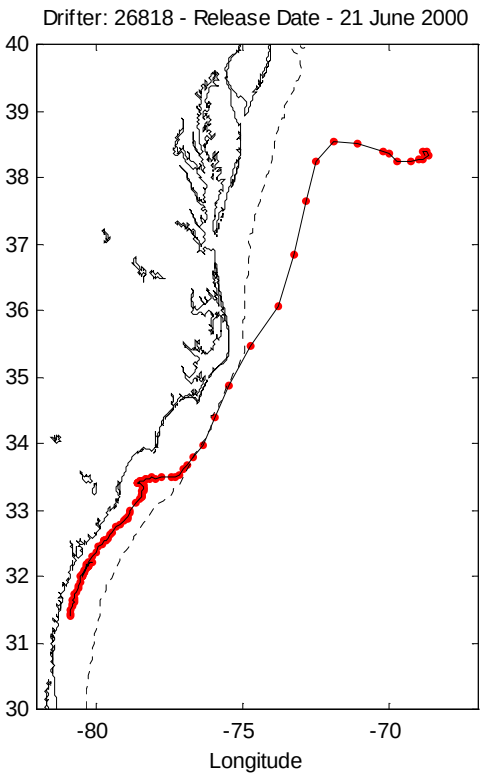

Figure 7. Track of a drifter released in June 2000. Shoreline and 50 m isobath are shown. Drifter was released within GRNMS.

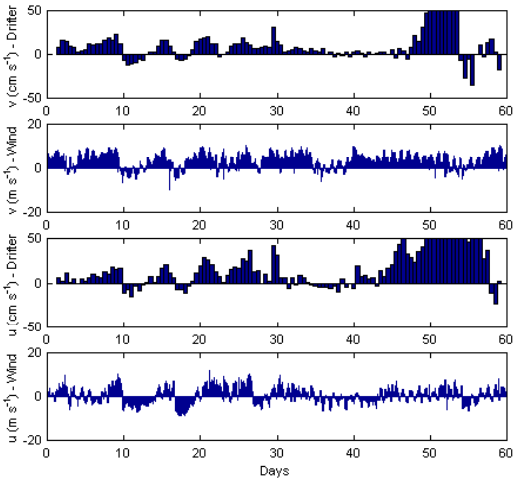

Figure 8. Comparison of north/south and east/west components of drifter 26818 movement with north/south and east/west component of the wind from NODC buoy located in GRNMS.

Provide an assessment of the efficacy of Grays Reef National Marine Sanctuary to act as a source of fish recruits for other hard bottom areas in the region

Preliminarily, the drifter data support the use of a wind-driven model to assess the fate of larvae spawned in the vicinity of GRNMS. Such models can be coupled with the data on which species are spawning within GRNMS and information on larval durations (to be derived from otolith analyses from fish collected in the beam trawl) to determine the areas to which GRNMS could provide larvae. Such an analysis would provide an initial assessment of the efficacy of Grays Reef National Marine Sanctuary to act as a source of fish recruits for other hard bottom areas in the region

Provide an assessment of the condition of macroinfaunal assemblages, concentrations of chemical contaminants in sediments, and contaminant body-burdens in target benthic species of the Gray's Reef National Marine Sanctuary

FY2000 sampling effort was completed during the week of April 3-7, 2000 on NOAA Ship FERREL Cruise FE-00-06-GR. At each of 20 random stations, samples and in-situ measurements were obtained for characterization of: (1) biodiversity and abundances of

macroinfauna (> 0.5 mm); (2) concentration of sediment contaminants (metals, pesticides, PCBs, PAHs); and (3) general habitat conditions (water depth, dissolved oxygen, salinity, pH, temperature, water clarity, % silt-clay versus sand content of sediment, organic-carbon content of sediment). The random sampling design used on this study will support statistical estimation of ecological condition throughout GRNMS with respect to these various measured parameters. Target benthic species of economic and ecological importance (black sea bass and molluscs) were collected from selected areas for analysis of contaminant levels in tissues. A cruise report of the samples that were collected and preliminary field observations was submitted in May 2000. Benthic infaunal and contaminant analyses are underway and will be completed during FY2001.

Biomarkers of Corals in GRNMS (preliminary objective title)

Oculina varicosa is a branching stony coral found along the Atlantic coast. We were able to obtain 10 fragments (approx 60-70cm length) during the July 1, 2000 cruise, with the assistance of Gray's Reef National Marine Sanctuary staff. These samples are being used to develop protocols for maintaining live corals in the laboratory and to develop biomarker methods used to evaluate stress and general health conditions in corals. We conducted three preliminary experiments with a few subsamples from some of the coral fragments and included lipid analysis, establishing primary cell cultures and a new biomarker assay for determining DNA damage, non-acridine orange exclusion assay.

Oculina varicosa, collected at GRNMS 1 July 2000, were maintained in aquaria (water temperature 20°C, salinity 36-37 ppt) under a 12 hr light/dark cycle of blue coral light and fed a diet of *Artemia* larvae supplemented with Phytoplex (marine phytoplankton supplement, Kent Marine), strontium and molybdenum (Figure 9). Water flow was maintained by placing power heads at opposite ends of the tank and operating them on an alternating on/off 12 hr cycle. Water was aerated with an airstone and filtration with a Magnum canister filter device. Water was changed twice weekly, by replacing half of the water and the corals fed Artemia larvae daily.

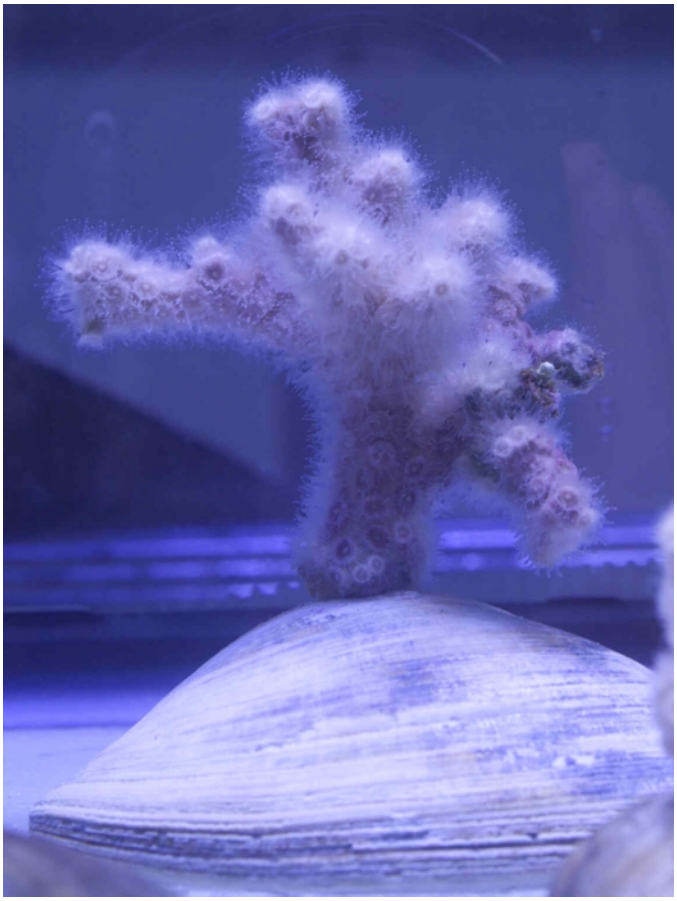

Figure 9. *Oculina varicosa* collected at GRNMS maintained in aquaria at the Center for Coastal Environmental Health and Biomolecular Research Charleston, South Carolina.

Little is know of the lipid composition and quantity in *Oculina varicosa* tissue and methods for such analysis of coral tissue are not readily available. We used sub-samples of GRNMS *O. varicosa* to conduct preliminary evaluations of lipid protocols as well as examine coral samples with and without heat stress to determine if there was any evidence changes in lipid composition or quantity. One sample was challenged with heat following an equilibration period of approximately one month, the water temperature of the one sample was raised to 31°C and held for 8 hours (heat-stressed). This heat-stressed sample and a control coral sample were used to evaluate applicability of routinely used lipid analytical methods to the extraction and quantification of total lipids, and qualitative and quantitative determination of lipid class and fatty acid composition of coral. We also tested and refined procedures for quantitative recovery of biomass and other techniques specific to the analysis of coral lipids. Since this was only an exploratory experiment, there were no replicates; therefore, we could not evaluate treatment effects.

Development of primary cell cultures was another of the effort.Coral tissue was enzymatically disrupted from the coral skeleton into a single cell suspension. These cells were washed and resuspended in Dulbecco's Modified Eagle's Medium in sterile sea water (36ppt) supplemented with L-glutamine and 2% fetal bovine serum, B-27 and antibiotics, plated in a 24-well tissue culture plate and maintained in a tissue culture incubator at 30°C. A variety of cell types were present in these primary cultures (Figure 10), notably neumatocysts and cells resembling assemblages of multicellular endothelial isolates (MEIs) from Acropora and *Pocillopora* species that have been described by Kopecky and Ostrander (Invitro Cell. Dev. Bio. –Animal 35:616-624, 1999). These MEIs continually spin in culture for several days. This primary mixed culture survived culture conditions for approximately 10 days. Cultures were terminated due to fungus contamination. Future attempts to establish a primary culture will include fungicides in addition to antibiotics.

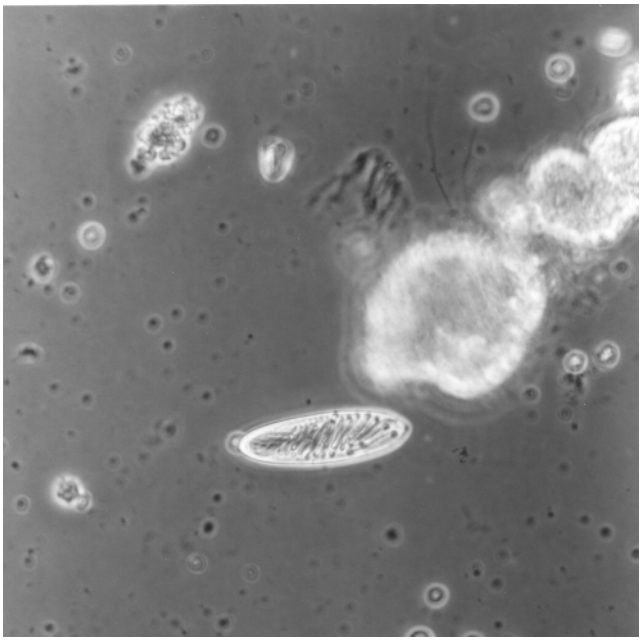

Figure 10. Photomicrograph of primary cell culture developed at Center for Coastal Environmental Health

Coral tissue was also used to explore the feasibility of using a human biomarker assay for detecting changes mitochondria, nonyl-acridine orange (NAO). Nonyl-acridine orange (NAO) is a fluorescent dye which binds to cardiolipin, a mitochondrial-specific lipid. NAO when excited with a 488 laser, will fluoresce in the green spectrum which is detected on a per cell basis using a flow cytometer. Changes in fluoresence is used to determine quantitative changes in mitochondria. If something is causing a loss of mitochondria, there is a

concomitant drop in the NAO retained by the cell. NAO should be retained irrespective of membrane potential, and just measure the amount of cardiolipin which is present in the cell. We test this new marker in corals to determine whether we could determine the volume/number of mitochondria and then use this to evaluate mitochondria during exposure to various stressors. Initial flow cytometer runs detected unacceptable levels of cellular debris, however, suggesting that the cryo-freezing technique was not appropriate for the coral cell preparation that was stained. We, however, believe that this is a valuable molecular biomarker and are continuing to conduct exploratory tests to determine how to optimally retain intact coral cells for this assay.

Other Activities

Jeff Hyland and Jon Hare participated in a State of the Reef Planning Meeting on March 21, 2000 in Charleston, SC.

Jon Hare presented the overall research plan and research update at the South Atlantic Fishery Management Council Habitat Advisory Panel on August 29-30, 2000 in Charleston, SC; Don Hoss and Jeff Govoni also attended.

Jon Hare made recommendations to Reed Bohne as to additional environmental measurements that could be collected at GRNMS in September, 2000.

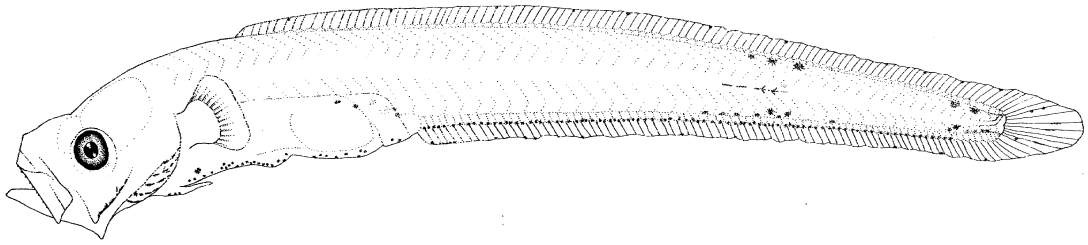

Figure 11. Larvae of Otophidium omostigmum, one of the most abundant Ophidiinae cusk eels collected in the vicinity of GRNMS

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